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A STUDY OF TRACE METALS FROM BARITES: THEIR CONCENTRATION, BIOAVAILABILITY, AND POTENTIAL FOR BIOACCUMULATION

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ABSTRACT

In recent years concern has been expressed by some regulatory authorities controlling offshore drilling operations regarding the potential for bioaccumulation in marine animals of the trace metals present in authorised discharges to the marine environment. One area of concern has been the naturally occurring trace metal concentrations present in all sources of barite used as a weighting agent in drilling fluids discharged along with drill cuttings.
Although barite bioavailability studies have previously been carried out, no definitive conclusion on effects has been reached. This has resulted in the US EPA setting limits (based on the precautionary principle) for levels of Mercury and Cadmium in barite used offshore in the Gulf of Mexico and in Alaska. In the North Sea area, PARCOM requires barite with the lowest levels of trace metals to be used for offshore drilling.
Following a UK Government/Industry working group that investigated this issue, a 60 day biological study using laboratory scale mesocosms, was carried out, based on the UK working group recommendations. The general findings and conclusions of this study are presented in this paper, along with associated analytical work that was carried out.

INTRODUCTION

Use of Barite in Drilling Fluids and other Industrial Uses

Some 4.75 million tonnes of barite was produced in 1995. The vast majority (80-90%) was consumed as a ground product by the oil well drilling industry as a weighting agent with lesser amounts serving the barium chemical, filler aggregate and radiation shielding markets. It is also used as a weighting agent in all drilling fluids, and is the most widely used mineral for this purpose due to its:-

- Availability as a naturally occurring mineral throughout the world.
- Ideal physical properties, principally; density, softness, and wettability.
- Being chemically inert.

Barite Sources

Barite occurs in a number of geological styles. Principle amongst these are bedded deposits, being layers of barite formed as a natural chemical sediment as part of a sedimentary or

volcanic sequence. In plan and section these deposits are lens shaped and exhibit good continuity, although they may become extensively deformed subsequent to deposition.
Of secondary importance individually but of equal importance in aggregate, are vein deposits, being infills of open ground in faults. Closely associated are cavern fill deposits in limestones. Deposits of vein type are characterised by pinch and swell discontinuity, whilst those of cavern type reflect the original irregular shape of the cavern in which they were deposited. These types of deposit were formerly extensively worked on a small scale by labour intensive methods, both underground and by openpit throughout Western Europe often in association with or subsequent to their exploitation for Lead. Elsewhere in the World where labour costs are low, such vein deposits continue to constitute major sources of supply to the international drilling fluids market.

The Mineralogy/Chemistry of Barite Ores

All barite ores contain traces of heavy metal bearing minerals in various proportions and extents. Even a perfect glassy transparent crystal of barite contains traces of many other elements other than those of the perfect chemical formula both stoichemetrically substituted in the lattice and also as solid and fluid inclusions. Other included minerals such as silica or barytocalcite are common, as are saline fluid inclusions. In nature there is no such thing as purity.
The contention made in the US EPA legislation that stratabound bedded deposits are "cleaner" with respect to heavy metals than vein deposits is incorrect. Each deposit has its own unique character, whilst metals levels vary across individual deposits. It is therefore dangerous to generalise along the lines that barite from a specific country or even a source is high/low in trace metals etc., although that might be true for one or a group of shipments. In cases in the third world where barite is marketed through a central selling agency there is always a question as to whether the barite in the next shipment will be sourced at the same mine or even in the same geological region.

REGULATORY CONTROL

For several years, PARCOM have instigated "programmes and measures" to both, monitor levels of pollutants (including trace metals) being discharged, and, require the best available techniques/best environmental practice to be used to minimise discharges.
In the absence of adequate environmental information detailing the effects following drilling discharges containing barite, regulatory authorities have tended to use the "Precautionary Principle" requesting that barites containing the lowest levels of trace metals be used offshore. Although what constitutes lowest levels has never been specified.
In the USA this approach has resulted in the EPA setting a maximum limit for levels of Mercury and Cadmium in barite supplied for offshore use at 1 and 3 mg/Kg respectively. These levels were set on the basis:-

1. That they represent the "cleanest" commercially available barite available (on the assumption 2 [below] is correct).
2. That barite with these low levels of Mercury and cadmium will also have the lowest levels of other associated metals (Chromium, Zinc Lead, etc This assertion is not supported by the facts and is incorrect.
3. Barite derived from "bedded" deposits contain less trace metals than barite from "vein" deposits. This assertion is also not supported by the facts and is incorrect.

The EPA will review these regulations when such barite is no longer commercially available, at which time the "industry standard" will be changed to accommodate the next "cleanest"

source available. The result is that it is becoming increasingly difficult to meet the "1 and 3" criteria on imports to the US markets.

THE STUDY

Method

A sixty day chronic marine sediment bioaccumulation study using laboratory scale mesocosms was carried out using the lugworm *Arenicola marina,* the clam *Scrobicularia plana* the brown shrimp *Crangon crangon* and turbot *Scophthalmus maximus*. *Arenicola* and *Scrobicularia* are both deposit feeders while *Crangon* and *Scophthalmus* live in intimate contact with the surface of the sediment. The study comprised four treatments (a "clean" sediment control and three sediment/barite mixtures) with three replicates of each treatment being used. A full characterisation of the metals (As, Cd, Cr, Cu, Hg, Pb, Ni, Zn) in the sediments and interstitial waters of each treatment (using sequential extraction and aqua regia digestion procedures) was carried out on day 0. Tissue samples from each species were collected on days, 0, 20, 40, and 60 and initially tissue metal levels were measured in the day 0, 20, and 60 samples. Measurements of the biological effects of the different sediment/barite treatments were also made, including cast production in the lugworms and growth in turbot, mortality in all species, and liver metallothionein content, growth and mortality in turbot (the protein has been shown to be induced in certain aquatic organisms following exposure to the heavy metals cadmium, copper and zinc). A repeat sixty day study using only *Crangon crangon* was also carried out due to the complete mortality of these organisms in the initial study by day 40.

The study objectives were to:

1. Determine the chemical speciation of trace metals in the different sediment/barite treatments.
2. Assess the bioaccumulation and toxic effects of trace heavy metals from sediment/barite combinations on the following marine organisms; *Arenicola marina*, *Scrobicularia plana, Crangon crangon* and *Scophthalmus maximus*.

Establish relationships, where possible, between the different heavy metal species present in barite and organism responses, such as body loadings/cast formation in lugworms, metallothionein levels in shrimps and fish/fish growth, and mortality in all species.

METHODS AND MATERIALS

Selection of Barite Sources for Study

Barites representing three sources of clearly differentiated mineralogy and metals content were selected. One was representative of barite typically supplied for the UK/North Sea market, the two others representing both, the lowest possible, and high levels of trace metals in barite. The latter two are not commercially supplied for use in drilling fluids, the barite with lowest metals being medical grade barite, and the other is not available in sufficient quantities.

To summarise, the samples selected were:-

1. Medical Grade barite	Representing barite with the lowest metals concentrations.
2. North Sea barite	Representing barite with typical metals concentrations.
3. High Metals source	Representing barite with high metals concentrations.

The medical grade barite was supplied ready ground, the other two samples were supplied as 1 crushed material which was subsequently ground to nominal API particle size specification in a metal free grinding process at Imperial College London.

PREPARATION OF THE TEST TREATMENTS

Test sediment

The 'clean' control sediment was obtained from Newton Bay, Poole This sediment (based on dry weights) had a particle size distribution of 6% coarse sand, 68.9% medium sand, 20.1% fine sand, 1.7% very fine sand and 2.6% silt/clay and an organic matter content of 0.41% which is similar to that found around a North Sea drilling platform at the Bremerhaven workshop on sediment toxicity (Chapman *et al.* 1992).

Seawater

The seawater used to maintain test organisms prior to testing and in the test itself was obtained from two sources: MAFF, Lowestoft and Copine Fisheries, Portland. Each new load of seawater was analysed for heavy metal content to ensure acceptable heavy metal levels (Environmental Quality Standards) were not exceeded.

Test species

The animals of each species used in the study were within a narrow size range since metal contents in the tissues of several invertebrates have been shown to depend on organism size (Ray *et al.* 1980).

Preparation of the sediment/barite treatments

Six replicates of each sediment/barite treatment and six controls (sediment only) were used in the study, which comprises 24 treatments in total. Each treatment was replicated 6 times and each replicate consisted of large polyethylene tanks (102 x 69 x 60 cm internal dimensions). Three of the 6 tanks for each treatment contained the lugworms and clams, while the other 3 tanks were divided into two and contained the shrimps and turbot. The lugworms and clams were divided up between 3 small plastic tanks (40 x 30 x 12 cm external dimensions) which were modified so that interstitial water could exchange between the sediment within the tanks and the surrounding sediment. The tanks were set up using a computer generated random number sequence.
Each barite type was diluted with the control sediment at a ratio of 1:20 using 20 kg aliquots of each barite source and 380 kg of control sediment. This procedure resulted in a sufficient amount of each sediment/barite mixture to fill the relevant experimental tanks to a depth of approximately 10 cm.

Pre-test procedure

The seawater flow-through system was started seven days prior to addition of the test organisms to establish an redox potential (E_h) equilibrium in each tank. The E_h was then monitored daily at depths of 1 and 6-8 cm using a redox probe and meter.
The tanks were drained down to 1 cm of overlying water at the start of the experiment in order to take samples of the porewater. 'oxic' porewater was removed from the top 1 cm of the sediment and 'anoxic' porewater from a depth of 6-8 cm.
On day 0 before addition of the test organisms samples (50 g) of the control sediment and sediment/barite treatments from each replicate were taken from the upper layer (1 cm depth) and the lower layer (6-8 cm depth) and bulked. These samples were subjected to sequential

extraction to obtain the exchangeable, carbonate, reducible, organic and residual phases. The resulting extracts were analysed for concentrations of arsenic, cadmium, chromium, copper, lead, mercury, nickel and zinc using the procedures given in Gunn *et al.* (1989).
The test was conducted in an environment with a natural photoperiod (approximately 16 hours light and 8 hours dark) and a water temperature maintained around 15 °C using cooling coils placed in a storage tank supplying the experimental tanks.

Addition of test organisms

On day 0 of the study groups of 48 lugworms and 18 clams and 35 shrimps and 35 turbot were added to the appropriate replicates of the controls and sediment/ barite treatments, ensuring an initial even distribution within the tanks.
Sampling and monitoring procedures
Monitoring procedures
The following water quality and sediment quality measurements were taken weekly: the redox potential of the sediment (at 1 cm and 6-8 cm depths) and the dissolved oxygen concentration (DO) of the overlying water in each tank. The temperature and salinity of the overlying water was measured daily for the duration of the study. On day 60 sediment samples were taken from 1 cm and 6-8 cm depth in the replicate tanks of each treatment. These samples were then bulked and analysed for heavy metals concentrations using aqua regia digestion.

Tissue sampling

On day 0, 20, 40 and 60 of the study groups of lugworms (n=16), clams(n=6), shrimp (n=6) and turbot(n=6) were removed from each of the three experimental replicates of a treatment for heavy metal analysis (arsenic, cadmium, chromium, copper, lead, mercury, nickel and zinc). Whole lugworms, the soft tissues from clams, tail muscle from shrimp and gill, liver and muscle tissue from turbot were used. The tissues from a test species in each treatment replicate were rinsed with distilled water, pooled, weighed and placed in clean polypropylene tubes. The samples were stored at -18 °C in a freezer until analysed.
Lugworms and clams were removed from the relevant treatments and placed in tanks containing clean seawater and control sediment for 4 days before the animals were removed for tissue analysis. This depuration period was used based on a preliminary study in which clams were exposed to cadmium and zinc contaminated sediment for 14 days followed by transfer to the 'clean' sediment for 7 days. A depuration period of 4 days was required to ensure measured body concentrations in lugworms and clams from the sediment/ barite treatments were not elevated by the presence of contaminated sediment in the gut of these species. Clean sediment was provided in the depuration tanks since Bryan *et al.* (1985) advocated this approach for burrowing species notably polychaetes such as *Nereis diversicolor.*

Physico-chemical characterisation and sequential extraction of barite and sediment/barite samples

In this study sequential extractions of barite and sediment/barite samples were performed as described in Gunn *et al.* (1989). This procedure is based largely on the frequently used schemes for sediment analysis developed by Tessier *et al.* (1979) and Rapin and Förstner (1983). The samples were extracted sequentially by the use of progressively stronger reagents whilst attempting to minimise overlaps in the metal species extracted by each fraction. In the procedure five fractions were extracted:

1. Exchangeable fraction (designed to identify metals which are weakly held to particles and are readily exchanged) - obtained by shaking the sample at room temperature with 30 ml of 1 M ammonium acetate/0.25 M calcium at pH 6 (acetic acid) for 3 hours.

2. Carbonate fraction (designed to identify metals which are bound to carbonates) - obtained by shaking the solid sample remaining from extraction 1 at room temperature for 5 hours with 20 ml of 1 M sodium acetate adjusted to pH 5 with acetic acid.

3. Reducible (moderately) fraction (designed to identify metals which are bound to iron and manganese oxides) - obtained by digesting the solid sample remaining from extraction 2 at 96 °C with occasional agitation for 6 hours with 30 ml 0.1 M hydroxylamine hydrochloride in 25% (v/v) acetic acid.

4. Organic fraction (designed to identify metals associated with residual organics and sulphides) - obtained by extracting the solid sample remaining from extraction 3 at 85 °C for 2 hours with 5 ml of 0.02 M nitric acid and 5 ml 30% hydrogen peroxide (adjusted to pH 2 with nitric acid), then continued extraction for a further 3 hours after the addition of 5 ml of 30% hydrogen peroxide. After cooling, there was an additional 30 minute room temperature extraction with 10 ml of 3.2 M ammonium acetate in 20% (v/v) nitric acid. The ammonium acetate was added to prevent adsorption of extracted metals onto the oxidised sediment.

5. Acid-extractable (residual) fraction (designed to identify the remaining metals) - obtained by digesting the solid sample remaining from extraction 4 for 4 hours at 120 °C with 20 ml of concentrated nitric acid.

After each stage, the residues were separated from the extracts by centrifugation and washed with de-ionised water. The combined extracts and washings were then acidified and made up to volume prior to analysis. However, there is some debate as to whether steps 4 and 5 have geological/mineralogical meaning particularly as 5 does not constitute a total digestion. This would require perchloric/Hydrofluoric acid or a perborate fusion followed by acid digestion.

Extraction of animal tissue

Animal tissue was prepared for mercury analysis by treating the material with a high temperature digestion of nitric and sulphuric acid in the presence of vanadium pentoxide. Arsenic in tissues was extracted using a magnesium nitrate and nitric acid digestion procedure. The tissue samples for cadmium, chromium, copper, lead, nickel and zinc determinations were digested by prolonged boiling in nitric acid. The digests were then made up to volume before analysis (Boumans *et al.* 1987).

BIOLOGICAL EFFECTS MEASUREMENTS

Determination of metallothionein concentration

Tissue metallothionein content was measured as an index of biological active cadmium, copper and zinc. In each sediment/barite mixture and the sediment-only control, metallothionein content was measured on day 60 in 3 hepatopancreas samples, each prepared from 5 *Crangon,* and on day 60 in 3 liver samples, each prepared from 5 *Scophthalmus*.
Differential pulse polarography was used to measure metallothionein protein concentration directly since this allows the rapid detection of minimal amounts of the heat stable protein in unpurified, heat-treated extracts (Bebianno and Langston 1990). The concentration of

metallothionein in each sample was calculated from the peak heights and expressed in mg g^{-1} dry weight of tissue homogenised.

Lugworm (*Arenicola*) cast formation

The lugworm *Arenicola* feeds on sediments and, when it defecates, produces characteristic "casts" on the sediment surface. Measurements of casts at frequent intervals can provide an indication of the health of the organisms (Matthiessen and Thain 1989). In the study, the surface of each replicate of the sediment/barite treatments containing lugworms was smoothed out every 7 days and the number of casts produced in the following 24 hours was recorded.

Growth of turbot

The weight of the surviving turbot in each sediment/barite treatment were measured on day 60 of the study. Since the fish were randomly added to the treatments at the start of the tests and there was a similar weight range of animals in each tank any subsequent differences in weight would provide an indication of the effects of the sediment/barite treatments on the growth of the fish.

Mortality of test organisms

In the study dead organisms of any species were removed and recorded whenever observed.

Repeated brown shrimp (*Crangon crangon*) bioaccumulation study

In the initial bioaccumulation study the brown shrimp in all the treatments (including the controls) had died before day 40 probably as a result of a combination of starvation and cannibalism. Therefore it was necessary to repeat this phase of the study to ensure data was available for all the test species. The repeated study was started immediately following the completion of the initial study and was conducted in the tanks which had contained the shrimps and turbot in the initial study. Following consultation with MAFF, the shrimps were individually contained in 15 cm circular mesh enclosures. Eighteen shrimps were used in each replicate of a treatment and these were fed *ad libitum* each day with live mussels (*Mytilus edulis*). The rerun of the *Crangon* bioaccumulation study was conducted in an environment with a photoperiod was 16 hours light, 8 hours dark, with a 30 minute simulation of dawn and dusk.

RESULTS AND DISCUSSION

Metal bioavailability

The potential for heavy metals from the barite treatments used in this study to bioaccumulate in test species is dependent on their bioavailability in the sediments. Consequently this study attempted to relate sequential extraction data on the heavy metals in the sediment/barite treatments to measured body loadings and biological responses of test organisms.

Barite characterisation

In the raw barite samples the total metal loadings based on aqua regia digests were <1.9 µg g^{-1} for barite 1, 8070 µg g^{-1} for barite 2 and 22 733 µg g^{-1} for barite 3. Table 1.0 shows the percentage contributions of each metal to the total metal loading of the barite

sample based on aqua-regia digests. In barite 1 chromium was the major component (50.3%) whereas in barite 2 zinc (66.4%) and lead (32.8%) were the major components. In barite 3 lead (96.6%) was the major component.

Table 1.0 Percentage contribution of each metal to the total metal loading of each barite sample based on aqua regia digests

Metal	Percentage contribution of each metal to the total metal loading of each barite sample (%)		
	Barite 1	Barite 2	Barite 3
Arsenic	1.7	0.2	<0.01
Cadmium	2.6	0.1	0.02
Chromium	50.3	0.03	0.02
Copper	13.1	0.3	0.1
Lead	10.5	32.8	96.6
Mercury	0.5	0.1	<0.01
Nickel	9.9	0.02	0.03
Zinc	11.5	66.4	3.2

The barite samples were also analysed by sequential extraction and the concentrations of the different metals in the three samples measured by the two procedures were highly comparable. Small differences (<5 times) were evident between total concentrations of arsenic, nickel and zinc for the barite samples when analysed by sequential extraction and aqua regia digestion procedures. The lower concentrations measured by aqua regia digests for arsenic and nickel were probably due to the inability of the procedure to extract certain components from the samples. Both the sequential extraction and aqua digestion procedures probably did not measure all the total metals available from the barite samples. The sequential extraction data showed the zinc concentrations in barite 2 were associated mainly with the residual and organic fractions (organics + sulphides) while lead was found predominantly in the residual, carbonate and reducible fractions. In barite 3 lead was associated mainly with the reducible fraction but also the organic and exchangeable fractions.

Table 2.0 Sequential extraction phases with which the major metal loadings in the barite 2 and 3 treatments were mainly associated on day 0 (after 7 days for equilibrium)

Treatment	Metal	Layer	Sequential extraction phase with which the metal was mainly associated
Barite 2	Lead	1 cm	Exchangeable, Reducible
		6-8 cm	Reducible, Residual
	Zinc	1 cm/6-8 cm	Residual, Organic
Barite 3	Lead	1 cm	Organic, Exchangeable
		6-8 cm	Organic, Reducible, Exchangeable

In the barite 2 treatment 35% of the total lead content in the 1 cm layer was found in the exchangeable phase of the 1 cm layer compared with only 3% in the 6-8 cm layer, which is

the level found in this fraction in the raw barite. This association in the 1 cm layer with the exchangeable fraction is reflected in the higher lead concentrations in 1 cm layer porewaters compared to the 6-8 cm layer porewaters (Table 2.0). This may have been the result of diffusion of lead up from the 6-8 cm sediment via the porewater followed by adsorption in the 1 cm layer.

For the barite 3 treatment there was no significant increase in the exchangeable lead from the raw material and it appears to have transferred from the reducible phase in the native barite (47.9% of the total) to the organic phase (organic/sulphide) in the sediment/barite treatment (44.0% of the total). However, the high lead concentrations in barite 3 still resulted in high lead concentrations in the exchangeable fraction and high levels in both the 1 cm and 6-8 cm porewaters relative to the controls (Table 2.0). Slightly elevated levels of cadmium and copper were also observed in the porewater of barite 3 treatments, relative to the control.

On this basis at the start of the study the lead in the 1 cm and 6-8 cm sediment layers of the barite 2 and 3 treatments would be potentially bioavailable to test organisms. The high zinc concentrations in the upper and particularly the lower layers of the barite 2 treatment (relative to the controls) would be less available due to the high levels associated with the residual fraction.

On day 60 the aqua-regia digests on the upper and lower sediments showed that the concentrations of most metals (including lead and zinc) were higher than the totals obtained from the digestions carried out at the start of the experiment. The higher concentrations in samples taken on day 60 may have resulted from either:

the samples containing a higher proportion of barite and hence a higher metal content possibly due to a form of sediment redistribution associated with bioturbation. In the day 60 analysis of the sediment/ barite treatments the 1 cm layer samples, almost in all cases, had the greater concentrations of metals. This finding is an exact reversal of the samples taken on day 0 where the 6-8 cm layer samples were higher, and would probably be related to the particle redistribution and migration upwards of the barite.

additional metals becoming available to the extraction methods due to equilibrium reactions occurring in the sediment/barite treatments over time. These reactions could include the partitioning of the high concentrations of metals found on day 0 in the porewater onto the sediment over time as a result of an equilibrium being achieved.

Table 3.0 summarises the metals which were significantly elevated in test organisms (relative to controls) after 60 days exposure to the different sediment/barite treatments.

Table 3.0 Summary of the statistically significant increases in tissue metal levels measured in the test organisms in the different sediment/barite treatments after 60 days

Treatment	Metal	Tissues in which metal levels were elevated compared to the controls after 60 days
Barite 1	Arsenic	Lugworms
	Nickel	Lugworms
Barite 2	Copper	Lugworms
	Lead	Lugworms, Clams
	Zinc	Lugworms
Barite 3	Arsenic	Clams
	Cadmium	Turbot liver
	Lead	Clams, Brown shrimp tail muscle, Turbot gills

The increases in lead concentrations in test species (particularly lugworms and clams) exposed to the barite 2 and 3 treatments increased from day 20 to day 60 and may not have reached a threshold concentration. Therefore tissue samples from each test species taken on day 40 were subsequently analysed for lead to ascertain whether a threshold concentration had been reached in the different treatments after 60 days exposure. Generally lead concentrations in the tissues reached a maximum concentration after 40 days and either remained at that level or declined over the next 20 days (that is to day 60). The only apparent exceptions to this finding were the data for lugworms in barite 2 and clams in the barite 2 and 3 treatments. Analysis of variance and Tukey tests showed that the differences in lead levels in lugworms and clams between days 40 and 60 were not statistically significant ($P>0.05$ in all cases).

Comparison of measured tissue metal concentrations with literature values

In most cases the extent of the barite-induced increases in tissue metal concentrations which were statistically significant were between 16-37% higher than control values. The only exceptions were the increases in lead recorded in lugworms, clams, brown shrimps (tail muscle) and turbot (gills) which all exceeded 1100%. To realistically assess the potential impact of the barite treatments on aquatic organisms it is important to compare these statistically significant changes in tissue concentrations with published data for body burden-toxicity relationships and levels in test organisms from clean and contaminated conditions. There is a paucity of body burden-toxicity data for lead in the test species, therefore, comparisons have focused on information on heavy metal loadings in the species from clean and contaminated sites.

For lugworms, the statistically significantly elevated tissue arsenic (10.88 $\mu g\ g^{-1}$) and nickel (8.40 $\mu g\ g^{-1}$) levels recorded in animals from the barite 1 treatment and copper (3.91 $\mu g\ g^{-1}$) and zinc (19.7 $\mu g\ g^{-1}$) in the barite 2 treatment were consistent with tissue levels of these metals recorded by Jenner and Bowmer (1990) in lugworms from a clean site (12.5 μg As g^{-1}, 3.8 μg Ni g^{-1}, 3.8 μg Cu g^{-1} and 22.5 μg Zn g^{-1} respectively). However, the increased tissue lead levels measured in lugworms from the barite 2 treatment were higher than levels found in this species from contaminated sites around the coast of Wales (Packer *et al.* 1980).

Clams showed statistically significant increases in arsenic in the barite 3 treatment but the maximum arsenic levels recorded (5.65 $\mu g\ g^{-1}$) were consistent with the level reported in clams from clean sites (~4 $\mu g\ g^{-1}$) (Langston 1985). In contrast, the levels of lead found in clams from the barite 2 and 3 treatments were consistent with levels found in clams from contaminated estuarine sites (Bryan and Langston 1992).

The statistically significantly elevated concentrations of lead in the tail muscle of brown shrimps exposed to the barite 3 treatment (2.98 $\mu g\ g^{-1}$) were higher than those from *Crangon* exposed to contaminated sediment (0.95 $\mu g\ g^{-1}$) (Ray *et al.* 1981).

For turbot, the elevated gill cadmium levels in fish from the barite 3 treatment (0.018 $\mu g\ g^{-1}$) did not exceed levels (0.22 $\mu g\ g^{-1}$) measured by Nielsen and Bjerregaard (1991) in the gills of farmed juvenile turbot. No published data on levels of lead in the gills of turbot from clean and contaminated sites could be identified to compare with the elevated levels found in fish exposed to the barite 3 treatment.

On the basis of comparisons between tissue metal levels which were statistically significantly elevated compared to controls in this study and published data for levels in test species from clean and contaminated sites it would appear that lead is the only metal accumulating to levels typical of contaminated sites. Lead is not a required trace metal for any of the test organisms and accumulation by marine animals will ultimately result in toxic effects once tissue levels have exceeded a certain threshold level. This threshold level is species

dependent and will be determined by the extent of the detoxification mechanisms available to a particular species.

Biological effects measurements

Analysis of tissue metal concentrations in the test species exposed to the different sediment/barite treatments has shown that lead would appear to be the only substance likely to cause biological effects. On the basis of the levels of lead accumulated these should only potentially have occurred in barite treatments 2 and 3.
High levels of mortality (and low cast production) were found in lugworms in the barite 1 treatment but were not due to accumulation of heavy metals, An explanation as to the cause is not evident. In the barite 2 treatment the elevated tissue lead loadings did not result in any increase in mortality of test species or reductions in cast production in lugworms and growth in turbot. The high mortalities and reduced growth of turbot found in the barite 3 treatment may have resulted from the accumulation of lead. Despite accumulating high levels of lead the clams did not show increased mortality.

Determination of metallothionein levels in brown shrimp and turbot

The method for determining metallothionein levels in tissue samples was validated with standards prepared from rabbit metallothionein obtained from Aldrich and produced reproducible results.
Table 4.0 shows the metallothionein content in the hepatopancreas of brown shrimps and the livers of turbot exposed to the different sediment/barite treatments. There was no barite-dependent increase in metallothionein content in the hepatopancreas of brown shrimp or the liver of turbot. The lack of an increase in metallothionein content in the tissues of either species in sediment/barite treatments is consistent with the absence of elevations of cadmium, copper or zinc in the tail muscle of brown shrimp and the livers of turbot above levels expected in organisms from 'clean' sites.

Table 4.0 Metallothionein content of the hepatopancreas of brown shrimp and the livers of turbot exposed to different sediment/barite treatments

Tissue	Metallothionein content (µg g^{-1} wet weight) in samples from different sediment/barite treatments			
	Control	Barite 1	Barite 2	Barite 3
Brown shrimp hepatopanceas	3.24	10.21	6.13	5.41
Turbot liver	24.5	19.6	25.0	29.2

The metallothionein content of the control fish livers was 7.6 x higher than control levels in the brown shrimp hepatopancreas and was of a similar magnitude to the value of 63.0 ± 10.3 µg g^{-1} (wet weight) reported by Overnell *et al.* (1988) in control turbot.

CONCLUSIONS

1. On day 0 of the study (after 7 days equilibration) only the concentrations of zinc and lead in the barite 2 treatment and lead and, to a lesser extent zinc, in the barite 3 treatment were elevated compared to the control. The levels of these metals in the lower layer (6-8

cm) were higher in all cases than those in the upper layer (1 cm). The lead present at both depths in the barite 2 and 3 treatments was associated with the exchangeable fraction and was considered to be potentially bioavailable to test organisms. The high zinc concentrations in the 1cm layer and particularly the 6-8 cm layer of the barite 2 treatment were considered to be less bioavailable due to the high levels associated with the residual fraction.

2. By day 60 the concentrations of most metals in the upper and lower layers of each treatment (as measured by aqua regia digestion) were higher than the totals measured at the start of the study. This could have resulted from sediment bioturbation, or, additional metals becoming available due to equilibrium reactions occurring in the sediment/barite treatments over time (this process is a continual equilibrium process between the sediment and porewater).
3. In the sediment/barite treatments the elevated levels of certain metals in the tissues of test organisms remained consistent with levels found in the same species at clean sites. Only lead concentrations in the tissues of lugworms and clams in the barite 2 treatment and clam, shrimp tail muscle and turbot gills in the barite 3 treatment were significantly elevated (relative to controls) to levels consistent with those found at contaminated sites. Lead concentrations in surviving lugworms and clams reached a plateau at 40 days and were not statistically different in day 60 samples. Lead levels in the tail muscle of shrimps and the gills of turbot reached a peak at day 40 and declined by day 60. The finding that lead was accumulating in test species in barite 2 and 3 treatments to levels typical of contaminated sites, was consistent with the bioavailability data for heavy metals in the treatments.
4. The lifestyle of the test organisms was important in determining the extent of the impact of the different barite treatments. Lugworms and clams, both, ingested the sediment and burrowed deeply, coming into contact with the porewater, showed statistically significant increases in tissue lead concentrations in both barite 2 and 3 treatments. In contrast, the brown shrimps and turbot which were mainly in contact with the surface sediment only, showed elevated tissue concentrations in the barite 3 treatment. Clams showed greater tolerance of the elevated tissue lead levels than lugworms.
5. In assessing these results, the physicochemical differences between the different sediment/barite treatments and combinations of barite bearing drill mud discharges and sediments which would be found in the North Sea means the experimental exposure regime can only be considered indicative of the situation in the field.

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